Madroño, Vol. 57, No. 2, pp. 95–105, 2010

# SPECIES BOUNDARIES IN *PYRROCOMA LIATRIFORMIS* AND *PYRROCOMA SCABERULA* (ASTERACEAE) BASED ON AFLP DATA

James F. Smith and Dusty N. Perkins
Department of Biological Sciences, MS1515, Boise State University, 1910 University Drive, Boise, ID, 83725 USA
jfsmith@boisestate.edu

Curtis R. Björk
Box 131 Clearwater, B.C., V0E 1N0, Canada

Gina Glenne
Western Colorado Field Office, 764 Horizon Drive, Building B, Grand Junction, CO, 81506-3946 USA

Abstract

Previous investigations into the morphology of *Pyrrocoma liatriformis sensu lato* in northern Idaho and adjacent Washington have revealed two distinct morphologies that correspond to their geographical ranges. These same populations and individuals have been analyzed using AFLP data. Over 400 loci were identified among all individuals using two sets of AFLP adaptors. The data are in agreement with the morphological data and separate the populations from the Snake River Canyon/Camas Prairie from those of the Palouse grasslands. Data clustering methodologies using both presence/absence data for all individuals and allele frequencies for each population produced similar results. We suggest the name *P. scaberula* be resurrected to encompass the populations from the Snake River Canyon and Camas Prairie.

Key Words: AFLP, Asteraceae, Idaho, *Pyrrocoma*, species boundaries, Washington.

Numerous species concepts have been proposed to unambiguously determine species boundaries (see Niklas 1997; Howard and Berlocher 1998; Wilson 1999; Coyne and Orr 2004; Sites and Marshall 2003, 2004, for more detailed summaries). Selecting a concept poses a challenge to biologists, particularly botanists, where relatively common gene flow among morphologically distinct populations seems to preclude the widespread adoption of the biological species concept (Burger 1975; Donoghue 1985; Mishler and Brandon 1987; Ellstrand et al. 1996).

Over the past 20 yr, molecular methods have provided systematists an additional means of assessing species concepts beyond morphological variability (Miller and Spooner 1999; Lopez et al. 1999; Duim et al. 2001; Parsons and Shaw 2001; Dawood et al. 2002; Wiens and Penkrot 2002; Richardson et al. 2003; Martínez-Ortega et al. 2004; Sites and Marshall 2004; Whittall et al. 2004; Garzón et al. 2005; Irwin et al. 2005; Pons et al. 2006; Suatoni et al. 2006; Roe and Sperling 2007; Manoko et al. 2007; Meudt and Clarke 2007; Guo et al. 2008). Molecular data allow a means to determine if populations are genetically distinct from each other, obtain estimates of gene flow between populations, and resolve if they are mutually monophyletic.

Assessing population variation to resolve taxonomic status by molecular genetic means can be done by a variety of methods including simple sequence repeats (SSRs or microsatellites, Akkaya et al. 1995), inter-simple sequence repeats (ISSRs; Smith and Bateman 2002), randomly amplified polymorphic DNA (RAPDs; Smith and Pham 1996), amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) and randomly amplified fingerprinting (RAFs; Waldron et al. 2002). Many studies have employed AFLPs to provide evidence that a single species should be divided into multiple species where morphological data were limiting or conflicting (Kardolus et al. 1998; Lopez et al. 1999; Mueller and Wolfenbarger 1999; Duim et al. 2001; Hedrén et al. 2001; Koopman et al. 2001; Bottini et al. 2002; Parsons and Shaw 2001; Richardson et al. 2003; Martínez-Ortega et al. 2004; Whittall et al. 2004; Garzón et al. 2005; Irwin et al. 2005; Manoko et al. 2007; Meudt and Clarke 2007; Roe and Sperling 2007; Travis et al. 2008). According to Becker et al. (1995) AFLP fragments are derived from throughout the genome and bands of identical size (co-migrating) are predominantly homologous in closely related organisms (Waugh et al. 1997; Rademaker et al. 2000). Herein, we examine the AFLP variation among populations of *Pyrrocoma liatriformis sensu lato*.

*Pyrrocoma liatriformis* E. Greene (syn. *Haplopappus liatriformis* (Greene) St. John) is an herbaceous perennial found in northern Idaho and adjacent Washington (Fig. 1). This taxon has

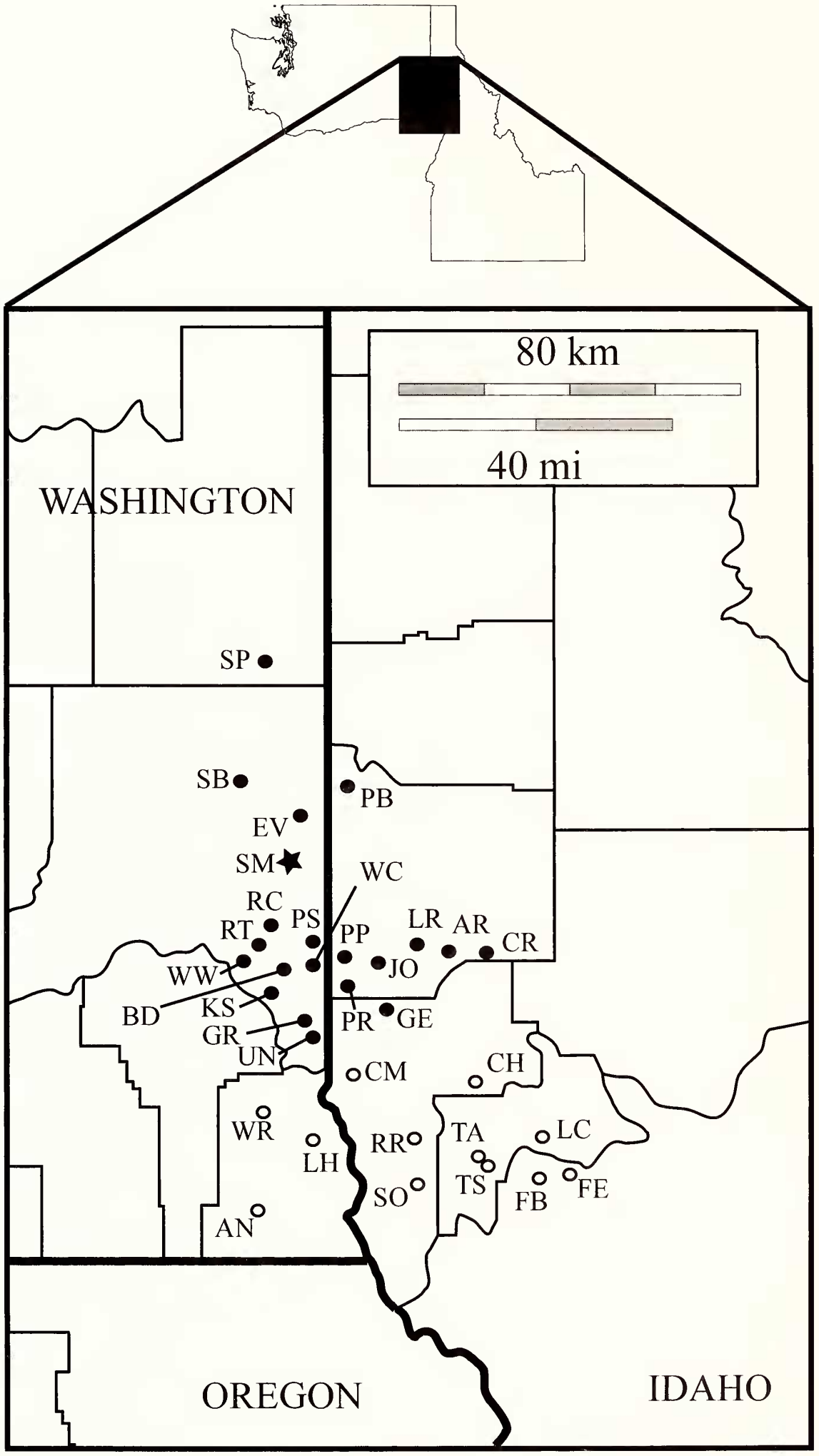


FIG. 1. Map showing the distribution of populations sampled in this analysis. Abbreviations follow Table 1. Open circles represent *P. scaberula* and closed circles represent *P. liatriformis*, the star represents *P. carthamoides*.

generally been viewed with a broad species concept (Hitchcock et al. 1955) that may encompass two distinct morphological species. Recent work on the morphology of *P. liatriformis sensu lato* has discovered that neither qualitative nor quantitative morphological characters are uniform across *P. liatriformis sensu lato* (Björk and Darrach 2009). Instead, the morphological variation is correlated with geographical range; plants from the Palouse grasslands are mostly

heavily tomentose throughout, resinous-punctate glands are absent and flower heads tend to be smaller (10–13.8 mm) whereas those of the canyon/Camas Prairie regions are hispid, with conspicuous resinous-punctate glands and flower heads are larger (13.8–15.1 mm). Taxonomic investigations of the type specimens of *Pyrrocoma* have revealed that these morphological entities have been described in the past as *P. scaberula* E. Greene (canyon/Camas Prairie plants) and *P. liatriformis* (Palouse grasslands); the former species largely has been considered a synonym of the latter in most treatments.

Björk and Darrach (2009) studied the morphological variation that separates *P. liatriformis* from *P. scaberula* by examining eight continuously variable morphological characters for 325 plants, 201 individuals in 18 populations from the Palouse and 124 individuals in 13 populations from the canyon/Camas Prairie region. The characters showed clear non-uniformity and although there is overlap in the ranges of the characters, the means of six characters (lateral branches, number of heads, head length, head width, phyllary width, and leaf width) are statistically significant for each group of populations. Furthermore, principle component analyses clearly separated the populations into two distinct groups. Although some populations in the Palouse grasslands share the lack of tomentum and strong glandularity of the canyon/Camas Prairie plants, they were clearly attributable to the morphology of *P. liatriformis sensu stricto* based on quantitative characters.

Despite the ability of the characters to separate the Palouse and canyon/Camas Prairie populations into distinct clusters, there is a strong overlap among the ranges of the morphological characters. These data suggest the two species are either closely related, represent a progenitor-derivative species pair (Gottlieb 1973, 1974; Gottlieb and Pilz 1976; Crawford and Smith 1982; Ranker and Schnabel 1986; Perron et al. 2000), undergo hybridization, or are a combination of these.

It is the goal of this study to resolve whether the morphological species as defined by Björk and Darrach (2009) are congruent with patterns shown by molecular data.

## Materials and Methods

To obtain an estimate of molecular genetic variability, we sampled 32 populations of *Pyrrocoma liatriformis sensu lato* from both Palouse grassland and canyon/Camas Prairie populations (Fig. 1). One additional population of *P. carthamoides* was used as an outgroup for comparison. Populations, their abbreviations, and tentative species identifications are presented in Table 1 and plotted onto a map in Fig. 1. These are the same populations and individuals sampled for morphological data by Björk and Darrach (2009). At the time sampling was conducted, exceptionally dry conditions in the region resulted in few populations that were flowering, which minimized the number of individuals that were sampled per population (Björk personal observation). Ideally 25–35 individuals were to have been sampled, but a total of 25 or more individuals was possible for only five of 33 populations (Table 1). Leaves were collected on silica gel and were the source of DNA extraction using DNeasy kits (Qiagen, Valencia, CA). One fertile stem per plant from populations of larger size was collected as a voucher. Plants from smaller populations were vouchered nondestructively with photographs taken with a ruler for scale. All vouchers, including photographic ones, are deposited at the University of Idaho Stillinger Herbarium (ID).

Approximately 500 ng of DNA from each individual was digested with *Mse*I and *Eco*RI while simultaneously annealing the *Mse*I and *Eco*RI adaptors at room temperature overnight. Annealing of the adaptors to the ends of the fragments alters the restriction site and precludes further digestion or need to perform reactions separately. To reduce the overall number of fragments amplified, and thus improve detection of homologous amplified fragments, a pre-selective amplification was performed using the primers *Eco*RI + A (5′-GACTGCGTACCAATTCA-3′) and *Mse*I + C (5′-GATGAGTCCTGAGTAAC-3′) and 1 μL of digested/ligated DNA. Pre-selective amplification was run with 20 cycles of denaturing at 94°C for 30 sec, annealing at 56°C for 1 min and extension at 72°C for 1 min. Products were diluted 1:20 with TE buffer and 3 μL of the dilution were used in selective amplification using either the A primer set, M-CAC (5′-GATGAGTCCTGAGTAACAC-3′) and labeled (with dye for Li-Cor system) E-ACT (5′-GACTGCGTACCAATTCACT-3′) or the T primer set, M-CTC (GATGAGTCCTGAGTAACTC-3′) and labeled E-ACC (5′-GACTGCGTACCAATTCACC-3′). Final selection amplification used an initial denaturation at 94°C for 2 min followed by 10 cycles of denaturation at 94°C for 20 sec, annealing at 66°C for 30 sec and extension at 72°C for 2 min. This was followed with 25 more cycles that differed only in reducing the annealing temperature to 56°C. Lastly there was a 30 min extension period.

Final AFLP products were separated on 6.5% polyacrylamide gels and visualized on a Li-Cor LongreadIR automated sequencer (Li-Cor Biotechnology Division, Lincoln, Nebraska). Molecular weight size standards were run on each end of each gel. Digital images of the gels were analyzed using Gene ImagIR (Li-Cor Biotech-

TABLE 1. LOCATIONS AND ABBREVIATIONS OF POPULATIONS SAMPLED IN THIS ANALYSIS, THEIR SPECIES DESIGNATION BASED ON MORPHOLOGICAL DATA, AND AMPLIFICATION SUCCESS FOR EACH SET OF AFLP ADAPTORS (A AND T). Vouchers are deposited at the University of Idaho Stillinger Herbarium (ID).

| Species designation | Population abbreviation (number of individuals sampled) | Number of individuals amplified: (A/T) |
|---|---|---|
| *P. scaberula*—Anatone, Asotin Co., WA | AN (12) | 12/3 |
| *P. scaberula*—Chesley Railroad, Lewis Co., ID | CH (25) | 21/24 |
| *P. scaberula*—Craig Mountain, Nez Perce Co., ID | CM (24) | 24/23 |
| *P. scaberula*—Ferdinand Butte, Idaho Co., ID | FB (25) | 22/24 |
| *P. scaberula*—Ferdinand east/Meadow Creek, Idaho Co., ID | FE (16) | 13/13 |
| *P. scaberula*—Lawyer Canyon, Lewis Co., ID | LC (12) | 11/12 |
| *P. scaberula*—Lime Hill, Asotin Co., WA | LH (25) | 22/25 |
| *P. scaberula*—Redbird Road, Nez Perce Co., ID | RR (25) | 23/25 |
| *P. scaberula*—Soldiers Meadow, Lewis Co., ID | SO (1) | 1/1 |
| *P. scaberula*—Talmacks North, Lewis Co., ID | TA (12) | 0/12 |
| *P. scaberula*—Upper Cold Spring Creek, Lewis Co. ID | TS (5) | 5/5 |
| *P. scaberula*—Weissenfels Ridge, Asotin Co., WA | WR (8) | 7/7 |
| *P. liatriformis*—American Ridge, Latah Co., ID | AR (16) | 16/15 |
| *P. liatriformis*—Barking Dog, Whitman Co., WA | BD (16) | 16/16 |
| *P. liatriformis*—Cedar Ridge, Latah Co., ID | CR (10) | 10/10 |
| *P. liatriformis*—Eden Valley, Whitman Co., WA | EV (7) | 6/7 |
| *P. liatriformis*—Genesee South, Nez Perce Co., ID | GE (4) | 3/3 |
| *P. liatriformis*—Gross Road, Whitman Co., WA | GR (11) | 10/10 |
| *P. liatriformis*—Joel, Latah Co., ID | JO (5) | 5/5 |
| *P. liatriformis*—Kramer Prairie, Whitman Co., WA | KS (19) | 19/18 |
| *P. liatriformis*—Lenville Road, Latah Co., ID | LR (11) | 10/11 |
| *P. liatriformis*—Mix Road, Latah Co., ID | PP (5) | 5/4 |
| *P. liatriformis*—Palmer Butte, Latah Co., ID | PB (5) | 5/5 |
| *P. liatriformis*—South end of Paradise Ridge, Latah Co., ID | PR (15) | 15/15 |
| *P. liatriformis*—Palouse Prairie Strip,Whitman Co. WA | PS (9) | 8/8 |
| *P. liatriformis*—Rose Creek, Whitman Co., WA | RC (5) | 5/5 |
| *P. liatriformis*—Armstrong Road, Whitman Co., WA | RT (6) | 3/2 |
| *P. liatriformis*—Steptoe Butte, Whitman Co., WA | SB (7) | 7/7 |
| *P. liatriformis*—Spaulding Road, Spokane Co., WA | SP (3) | 2/2 |
| *P. liatriformis*—Uniontown, Whitman Co., WA | UN (3) | 2/2 |
| *P. liatriformis*—Whelan Cemetery, Whitman Co., WA | WC (25) | 20/23 |
| *P. liatriformis*—Wawawai Grade, Whitman Co., WA | WW (21) | 20/20 |
| *P. carthamoides*—Smoot Hill, Whitman Co., WA | SM (23) | 23/22 |

nology Division, Lincoln, NE) to determine molecular weight designations for each fragment. Gel images were edited to ensure that fragments of identical size were correctly assigned the same weights. These data were exported using Gene Profiler (Scanalytics, Inc., Fairfax, VA) and fragments were assigned an allele designation based on which set of adaptors was used (herein designated as either the A or T set of alleles) and their molecular weight. Each individual was then scored for presence or absence of each allele.

Fragments greater than 600 bp and less than 49 bp were excluded from the AFLP analyses. Large fragments may be amplified with lower frequency during the process, as a result their consistency may be less reproducible and reliable. Similarly, homology of larger fragments becomes less probable since there are greater opportunities for insertions and deletions of DNA to alter fragment sizes between individuals. Smaller fragments were excluded due to potential difficulties in resolving fragment sizes.

Data were entered into MacClade (Maddison and Maddison 2000) and exported as a simple table. The table was modified using AFLPDAT (Ehrich 2006) to convert the files to formats usable in STRUCTURE (Pritchard et al. 2000; Falush et al. 2007) as well as to generate allele frequency data for each population.

There are many alternative approaches to analyze AFLP data to detect structure within the data set (Bonin et al. 2007). In general, these break down into two analyses: 1) analysis of bands directly (presence/absence) or 2) converting the band data into allele frequency data for each population. Both data types can then be used in an array of methodologies to detect diversity and structure within and among the sampled populations.

Here we opt to make use of both band data and allele frequency data to generate tree-based representations of the variation (Bonin et al. 2007). We make use of two methods to analyze the data for both band and allele frequency data:

Neighbor-joining (NJ) using Jaccard's genetic similarities (bands) or Nei's (1972) genetic distance and UPGMA. These analyses are among the most widely used methodologies for resolving species boundaries using AFLP data (Miller and Spooner 1999; Duim et al. 2001; Koopman et al. 2001; Parsons and Shaw 2001; Coulibaly et al. 2003; Jacoby et al. 2003; Richardson et al. 2003; Dehmer and Hammer 2004; Martínez-Ortega et al. 2004; Whittall et al. 2004; Garzón et al. 2005; Manoko et al. 2007; Guo et al. 2008).

A data matrix of presence/absence for all bands was directly imported into PAUP* (Swofford 2002) for NJ and UPGMA analyses. Allele frequencies were used to calculate population genetic distances that were then used to generate NJ and UPGMA trees in PHYLIP (version 3.67; Felsenstein 2007).

We used the Bayesian clustering method implemented in STRUCTURE 2.2 (Falush et al. 2007) to determine the optimal number of groups indicated by the data. Assuming all populations are in Hardy-Weinberg and linkage equilibria, this method assigns individuals to one of the pre-specified numbers of genetic clusters, $K$, using multi-locus genotypes and Markov Chain Monte Carlo sampling. We ran separate clustering simulations for all populations over a range of one to 40 clusters (i.e., $K$ = one to 40). Individuals with data missing for either the A or T set of alleles were removed from the analyses. This reduced some populations to few samples (only three individuals for AN) and resulted in TA being removed completely. Simulations were run assuming an ancestry model that incorporates admixture and correlated allele frequencies across loci for one million generations with a burn-in of 25,000 generations, sampling every 100 generations.

We compared posterior probabilities of $K$ from one to 40 clusters using the ad hoc statistic, $\Delta K$ (Evanno et al. 2005). This statistic has been shown to be a better estimator of structure in some data sets, especially those where homogeneous dispersal among populations cannot be assumed (Evanno et al. 2005; Travis et al. 2008). In such cases, a common pattern is for STRUCTURE to plateau near the true value of $K$, and then to continue increasing gradually. Evanno et al. (2005) showed that $\Delta K$ consistently returns a clear peak at the true value of $K$ under a variety of migration models. We chose a maximum $K$ of 40 because this exceeded the number of sampled populations.

## Results

Amplification of DNA was successful for nearly all individuals for both sets of AFLP adaptors, A and T (Table 1). A few individuals did not amplify well which is perhaps the effect of plant resin that inhibits the reactions. No individuals from the TA population were amplified for the A alleles and only three from AN were amplified fully for the T alleles. Some individuals were not amplified for either allele. Resin was detected in the precipitation stage of the DNA extraction for some individuals of these populations.

For several of the gels, bands that were distinctly different in size below 50 bp were classified as the same size by the software. Therefore the limitations of the software for resolving bands at this stage required us to eliminate these bands from the analyses. Scanning gels visually indicated that these fragment sizes tended to be consistent across nearly all individuals, therefore their exclusion was unlikely to affect the results whereas their inclusion may have indicated erroneous relationships among individuals and populations.

For the A set of adaptors, 245 alleles were scored for 373 individuals ranging from 569 to 50 bp in size. For the T set of adaptors, 177 alleles were scored for 387 individuals ranging from 581 to 50 bp in size. The complete data matrix had 407 individuals from 33 populations and 422 alleles.

The analyses that used presence/absence data for all individuals were sensitive to missing data. Individuals with missing data for either allele were either 1) clustered together regardless of population designation, or 2) in disparate parts of the tree (often included in populations that were neither morphologically or geographically similar, data not shown). Therefore we removed these samples (AN1-9, AR8, CH2, 11, 12, CM11, EV5, FB9, 12, KS8, LC12, LH6,7,18, LR11, MIX1, RCA5, RR4, 7, RT4, TAO1-12, WC3,6,19,20, 22,23,25, WW6, and 12) and re-ran the analyses. We also ran analyses of the A and T alleles separately to confirm the placement of the individuals excluded above in their respective population (data not shown).

The analyses based on allele frequencies divided all populations into two distinct groups corresponding to *Pyrrocoma scaberula* and *P. liatriformis* based on a priori designations by Björk and Darrach (2009; Fig. 2). *Pyrrocoma carthamoides* clustered within *P. liatriformis* with the UPGMA analysis (data not shown) because this analysis does not allow the outgroup to be specifically designated as such. The NJ tree in contrast (Fig. 2), results in two distinct and monophyletic groups each for *P. liatriformis* and *P. scaberula*.

There are clear clusters within each of these groups. Populations that cluster together in both the NJ and UPGMA trees within *P. scaberula* are AN/FB/SO/TS, FE/WR, and LC/CH/LH/RR/CM. Only population TA changes position between the analyses and is found close to CH/

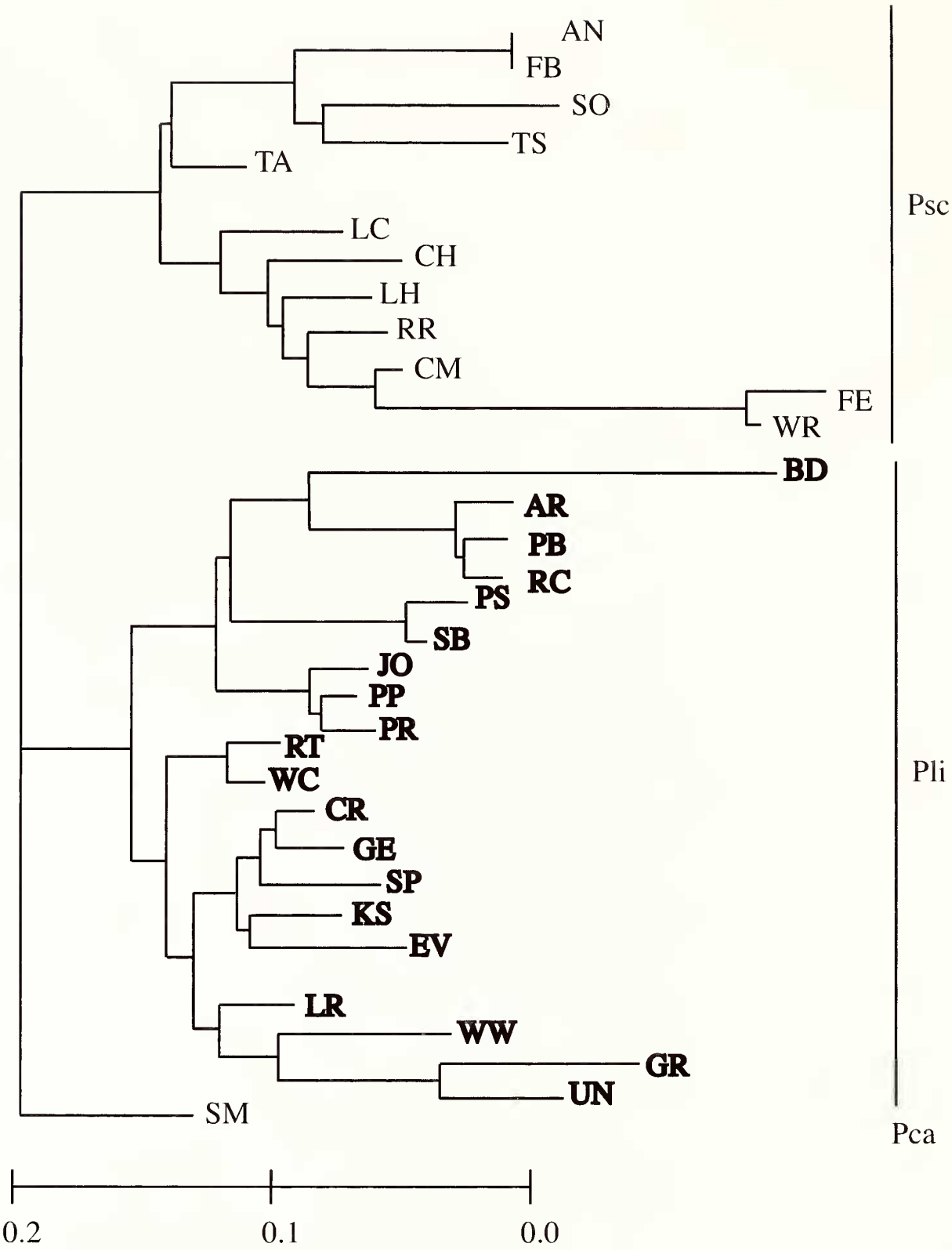


FIG. 2. Neighbor-joining based tree derived from AFLP allele frequency data. Population names follow abbreviations of Table 1. Bars to the right of the tree mark species boundaries, Psc—*Pyrrocoma scaberula*, Pli—*Pyrrocoma liatriformis*, Pca—*Pyrrocoma carthamoides*. Population names are abbreviated following Table 1 and are designated as normal font (*P. scaberula*) or bold (*P. liatriformis*).

CM/RR/LH/LC in the UPGMA analysis (data not shown). It should be noted that TA is lacking data for over half of the alleles. Within *P. liatriformis* there are similar clusters of populations that are consistent between analyses. These are AR/PB/RC, PS/SB, JO/PP/PR, RT/WC, GR/UN, and CR/GE/SP/KS/EV. The grouping of populations BD, LR and WW differ between the two analyses. With UPGMA, BD is close to all other populations of *P. liatriformis*, LR is close to RT/WC, and WW is close to CR/GE/SPKS/EVLR/RT/WC (data not shown).

The NJ analyses based on bands produced trees that were nearly identical to those based on allele frequencies (Figs. 2, 3) both in terms of clustering populations into species groups, and relationships of populations within each cluster. The greatest differences are that 1) not all populations were recovered as a single monophyletic group (Fig. 3), 2) the NJ tree did not result in a monophyletic *P. scaberula* due to the position of population LH, and 3) *P. carthamoides* is clustered within *P. scaberula* instead of *P. liatriformis* with the UPGMA tree (data not shown).

Groupings within each species are also similar to the frequency-based methods. The major differences within *P. scaberula* are the position of population LH in the NJ tree (Fig. 3) and that individual AN5 was more closely associated to

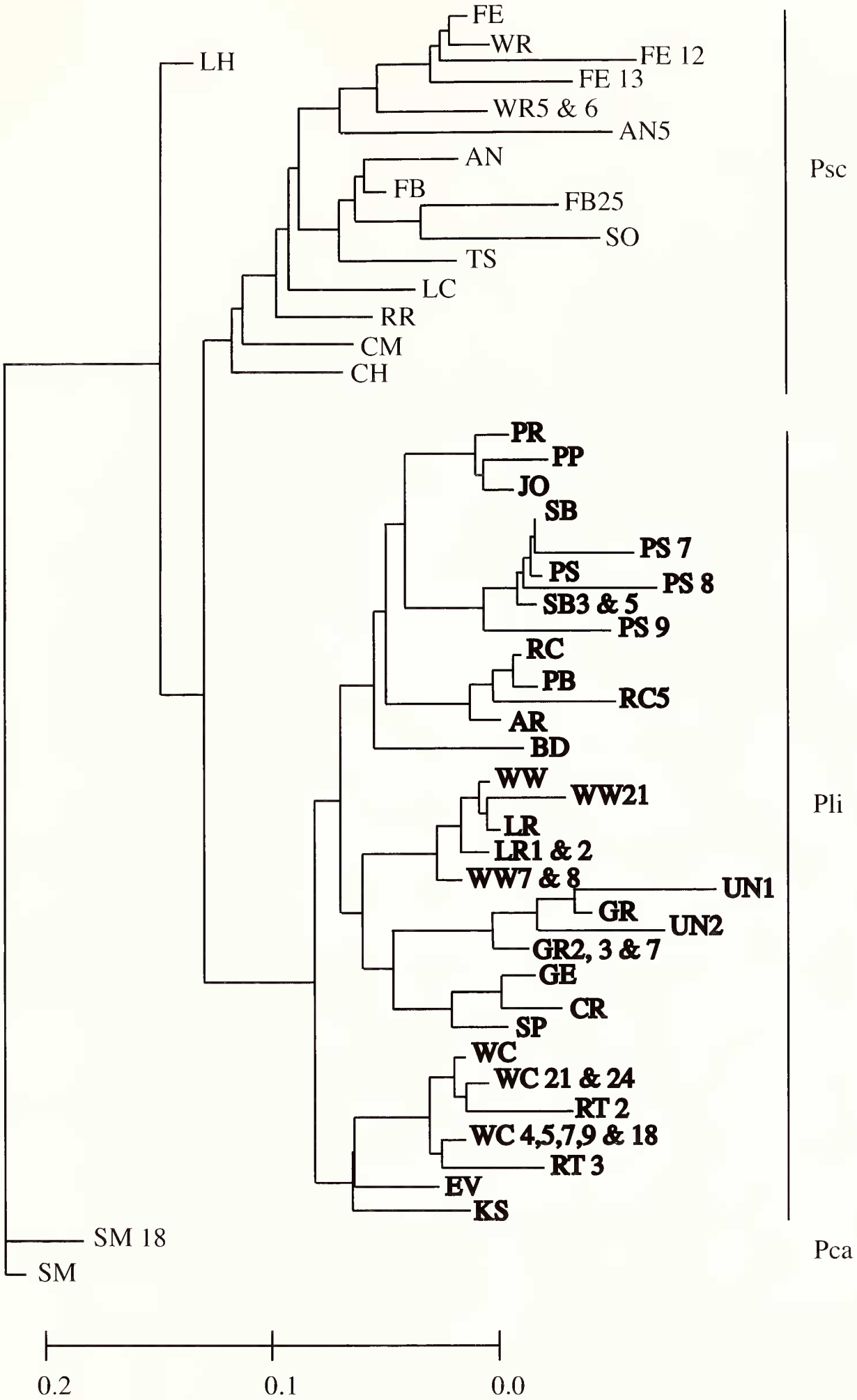


FIG. 3. Neighbor-joining based tree derived from AFLP band presence/absence. Where the majority of sampled individuals formed a single cluster only the population name abbreviation is used. In instances where individuals fell outside of their respective population cluster, they are designated with a population name and number for the individual. Population names are abbreviated following Table 1 and are designated as normal font (*P. scaberula*) or bold (*P. liatriformis*). Bars to the right of the tree mark species boundaries, Psc—*Pyrrocoma scaberula*, Pli—*Pyrrocoma liatriformis*, Pca—*Pyrrocoma carthamoides*.

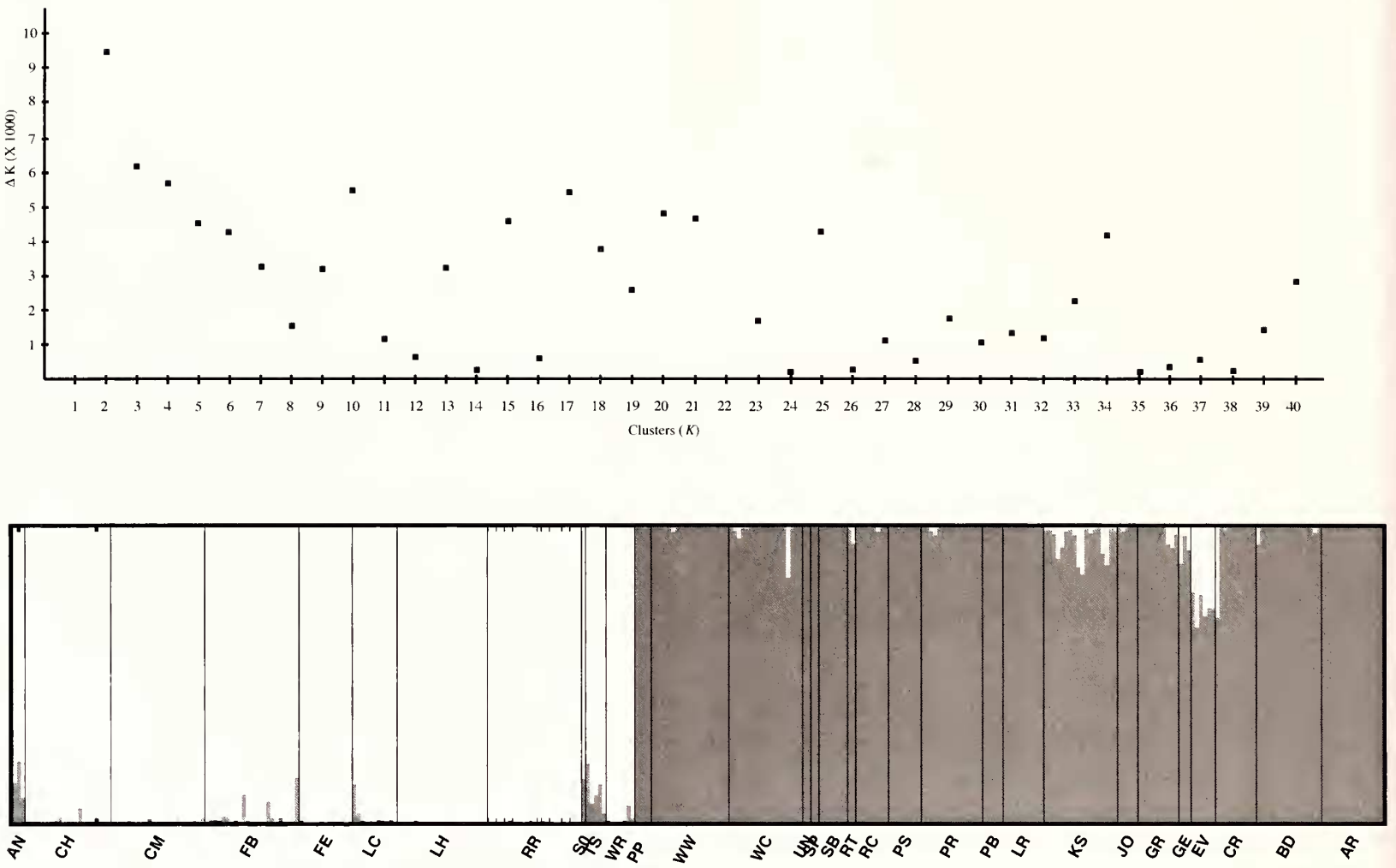


FIG. 4. Structure analysis of all populations of *Pyrrocoma liatriformis sensu lato* sampled in this analysis. A. Plot showing values of ΔK for each value of K. B. Bayesian assignment of individuals to two clusters. The bars represent the estimated posterior probabilities of each individual belonging to each of the two inferred clusters.

WR/FE than to FB/SO/TS. The lack of unity of population AN is likely due to the fact that only approximately half of the T alleles were scorable for AN5. Population TA was excluded from these analyses due to a complete lack of A alleles. Based on analysis that utilized only the T alleles, TA was close to AN/FB/SO/TS as it is in the NJ tree based on frequencies (Fig. 2). Likewise, groupings were similar within *P. liatriformis*, the main exceptions being populations KS and EV which showed a closer affinity to WC/RT based on bands (Fig. 3).

Plotting the actual values of $K$ from one to 40 indicated that there was a plateau after $K = 2$ with small increases in probability for each subsequent value of $K$. With all populations except the outgroup *P. carthamoides* included, the greatest $\Delta K$ was at 2 distinct clusters (Fig. 4). These results agree with the clustering results that divide the populations into two species. Only individuals of population EV show any significant probability of being assigned to the other species (Fig. 4).

## DISCUSSION

All analyses of AFLP data presented here, regardless of whether bands or frequencies were used, separate the populations into *Pyrrocoma liatriformis* and *P. scaberula* as determined by Björk and Darrach (2009) using morphological data (Figs. 2–4). The congruence of different methodologies is largely considered a means of overcoming potential problems of homology with AFLP data (Koopman et al. 2001) and the results of these analyses are congruent with previous work on morphology.

### Relationships Between Species

*Pyrrocoma scaberula* is paraphyletic based on NJ analysis of bands (Fig. 3). This raises the question whether these two species may or may not represent a progenitor-derivative pair (Gottlieb 1973, 1974; Gottlieb and Pilz 1976). The progenitor species would be expected to be paraphyletic since the derivative species would have resulted from a subset of populations. However, a second important criterion for a progenitor-derivative species is that the derivative species should contain a subset of the total diversity found in the progenitor. A summary of the presence/absence data shows that 73.2% of the alleles are shared between the two species, 15.7% are unique to *P. liatriformis* and 11.1% are unique to *P. scaberula*. These results indicate that a large portion of the data is shared among the individuals and populations rather than being unique to either the putative progenitor species (*P. scaberula*) or the putative derivative (*P.*

*liatriformis*) and thus argues against a progenitor-derivative pair. Likewise, the results of the Bayesian simulations in STRUCTURE do not indicate any overlap of populations between the two species, but instead the optimal data partition is equivalent to two groups (Fig. 4). It seems more likely that the paraphyly of *P. scaberula* is a result of recent common ancestry with shared alleles between the populations.

### Relationships of Populations within Species

The AFLP results clearly show population genetic structure within each species (Figs. 2, 3). Within *P. scaberula*, groups that consistently hold together following the tree-based methods include AN/FB/SO/TS/TA, FE/WR, and perhaps LC/LH/CH/RR/CM. Within *P. liatriformis*, AR/PB/RC, SB/PS, JO/PP/PR, GR/UN, CR/GE/SP, and RT/WC are commonly recovered. Populations BD, KS, WW, LR, and EV sometimes show relationships with other groups but not always. Population BD has the longest branches showing the greatest genetic distance from other populations. The results from STRUCTURE indicate that the populations are best treated as two clusters, corresponding to the two species. The one exception is population EV where individuals show the greatest probability of being assigned to *P. liatriformis*, but also have a noticeable probability of being assigned to *P. scaberula* (Fig. 4). These data may indicate some recent hybridization within this population, be the retention of a large number of alleles that are ancestral to both species, or reflect incomplete lineage sorting.

### Acknowledgments

We would like to thank Karen Gray and Tyson Kemper who offered their time to collect and send leaf material for the AFLP analyses and two reviewers and the editors of Madroño for improving this manuscript. We would also like to thank the United States Fish and Wildlife Service and Boise State University for providing funds to complete the AFLP analyses.

### Literature Cited

Akkaya, M. S., R. C. Shoemaker, J. E. Specht, A. A. Bhagwat, and P. B. Cregan. 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Science 35:1439–1445.

Becker, J., M. Kuiper, P. Vos, F. Salamini, and M. Heun. 1995. Combined mapping of AFLP and RFLP markers in barley. Molecular and General Genetics 249:65–73.

Björk, C. R. and M. Darrach. 2009. An investigation of morphological evidence supports the resurrection of *Pyrrocoma scaberula* (Asteraceae: Astereae). Journal of the Botanical Research Institute of Texas 3:231–238.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Molecular Ecology 16:3737–3758.

Bottini, M. C. J., A. De Bustos, N. Jouve, and L. Poggio. 2002. AFLP characterization of natural populations of *Berberis* (Berberidaceae) in Patagonia, Argentina. Plant Systematics and Evolution 231:133–142.

Burger, W. C. 1975. The species concept in *Quercus*. Taxon 24:45–50.

Coulibaly, S., R. S. Pasquet, R. Papa, and P. Gepts. 2003. AFLP analysis of the phenetic organisation and genetic diversity of *Vigna unguiculata* (L.) Walp. reveals extensive gene flow between wild and domesticated types. Theoretical and Applied Genetics 104:358–366.

Coyne, J. A. and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Crawford, D. J. and E. B. Smith. 1982. Allozyme variation in *Coreopsis neucensoides* and *C. neucensis* (Compositae), a progenitor-derivative species pair. Evolution 36:379–386.

Dawood, A., A. Channing, and J. P. Bogart. 2002. A molecular phylogeny of the frog genus *Tomopterna* in southern Africa: examining species boundaries with mitochondrial 12S rRNA sequence data. Molecular Phylogenetics and Evolution 22:407–413.

Dehmer, K. J. and K. Hammer. 2004. Taxonomic status and geographic provenance of germplasm acessions in *Solanum nigrum* L. complex: AFLP data. Genetic Research in Crop Evolution 51:551–558.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. The Bryologist 88:172–181.

Duim, B., P. A. R. Vandamme, A. Rigter, S. Laevens, J. R. Dijkstra, and J. A. Wagenaar. 2001. Differentiation of *Campylobacter* species by AFLP fingerprinting. Microbiology 147:2279–2737.

Ehrich, D. 2006. AFLPDAT: a collection of R functions for convenient handling of AFLP data. Molecular Ecology Notes 6:603–604.

Ellstrand, N. C., R. Whitkus, and L. C. Rieseberg. 1996. Distribution of spontaneous plant hybrids. Proceedings of the National Academy of Sciences USA 93:5090–5093.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611–2620.

Falush, D., M. Stevens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7:574–578.

Felsenstein, J. 2007. PHYLIP: Phylogeny Inference Package. Software and documentation distributed by the author, Seattle. WA. Website http://evolution.genetics.washington.edu/phylip.html [accessed 28 June 2010].

Garzón, C. D., D. M. Geiser, and G. W. Moorman. 2005. Diagnosis and population analysis of *Pythium* species using AFLP fingerprinting. Plant Disease 89:81–89.

Gottlieb, L. D. 1973. Genetic differentiation, sympatric speciation, and the origin of a diploid species of *Stephanomeria*. American Journal of Botany 60:545–553.

———. 1974. Genetic confirmation of the origin of *Clarkia lingulata*. Evolution 28:244–250.

——— AND G. PILZ. 1976. Genetic similarity between *Gaura longiflora* and its obligately outcrossing derivative *G. demareei*. Systematic Botany 1: 181–187.

GUO, Y.-P., J. SAUKEL, AND F. EHRENDORFER. 2008. AFLP trees versus scatterplots: evolution and phylogeography of the polyploid complex of *Achillea millefolium* agg. (Asteraceae). Taxon 57:153–169.

HEDRÉN, M., M. F. FAY, AND M. W. CHASE. 2001. Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). American Journal of Botany 88:1868–1880.

HITCHCOCK, C. L., A. CRONQUIST, M. OWNBEY, AND J. W. THOMPSON. 1955. Vascular plants of the Northwest. University of Washington Press, Seattle, WA.

HOWARD, D. J. AND S. H. BERLOCHER. 1998. Endless forms: species and speciation. Oxford University Press, New York, NY.

IRWIN, D. E., S. BENSCH, J. H. IRWIN, AND T. D. PRICE. 2005. Speciation by distance in a ring species. Science 307:414–416.

JACOBY, A., M. T. LABUSCHAGNE, AND C. D. VILJOEN. 2003. Genetic relationships between southern African *Solanum retroflexum* Dun. and other related species measured by morphological and DNA markers. Euphytica 32:109–113.

KARDOLUS, J. P., H. J. VAN ECK, AND R. G. VAN DEN BERG. 1998. The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanaceae). Plant Systematics and Evolution 210:87–103.

KOOPMAN, W. J. M., M. J. ZEVENBERGEN, AND R. G. VAN DEN BERG. 2001. Species relationships in *Lactuca* s. l. (Lactuceae, Asteraceae) inferred from AFLP fingerprints. American Journal of Botany 88:1881–1887.

LOPEZ, J. V., R. KERSANACH, S. A. REHNER, AND N. KNOWLTON. 1999. Molecular determination of species boundaries in corals: genetic analysis of the *Montastraea annularis* complex using amplified fragment length polymorphisms and a microsatellite marker. Biological Bulletin 196:80–93.

MADDISON, D. R. AND W. P. MADDISON. 2000. MacClade version 4.0. Sinauer Associates, Inc., Sunderland, MA.

MANOKO, M. L. K., R. G. VAN DEN BERG, R. M. C. FERON, G. M. VAN DER WEERDEN, AND C. MARIANI. 2007. AFLP markers support separation of *Solanum nodiflorum* from *Solanum americanum sensu stricto* (Solanaceae). Plant Systematics and Evolution 267:1–11.

MARTÍNEZ-ORTEGA, M. M., L. DELGADO, D. C. ALBACH, J. A. ELENA-ROSSELLÓ, AND E. RICO. 2004. Species boundaries and phylogeographic patterns in cryptic taxa inferred from AFLP markers: *Veronica* subgen. *Pentasepalae* (Scrophulariaceae) in the western Mediterranean. Systematic Botany 29:965–986.

MEUDT, H. M. AND A. C. CLARKE. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends in Plant Science 12:106–117.

MILLER, J. T. AND D. M. SPOONER. 1999. Collapse of species boundaries in the wild potato *Solanum brevicaule* complex (Solanaceae, *S.* sect. *Petota*): molecular data. Plant Systematics and Evolution 214:103–130.

MISHLER, B. D. AND R. N. BRANDON. 1987. Individuality, pluralism, and the phylogenetic species concept. Biology and Philosophy 2:397–414.

MUELLER, U. G. AND L. L. WOLFENBARGER. 1999. AFLP genotyping and fingerprinting. Trends in Ecology and Evolution 14:389–394.

NEI, M. 1972. Genetic distance between populations. The American Naturalist 106:283.

NIKLAS, K. J. 1997. The evolutionary biology of plants. The University of Chicago Press, Chicago, IL.

PARSONS, Y. M. AND K. L. SHAW. 2001. Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. Molecular Ecology 10:1765–1772.

PERRON, M., D. J. PERRY, C. ANDALO, AND J. BOUSQUET. 2000. Evidence from sequence-tagged-site markers of a recent progenitor-derivative species pair in conifers. Proceedings of the National Academy of Sciences USA 97:11331–11336.

PONS, J., T. G. BARRACLOUGH, J. GOMEZ-ZURITA, A. CARDOSO, D. P. DURAN, S. HAZELL, S. KAMOUN, W. D. SUMLIN, AND A. P. VOGLER. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 55:595–609.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

RADEMAKER, J. L. W., B. HOSTE, F. J. LOUWS, K. KERSTERS, J. SWINGS, L. VAUTERIN, P. VAUTERIN, AND F. J. DE BRUIJN. 2000. Comparison of AFLP and rep-PCR genome fingerprinting with DNA-DNA homology studies: *Xanthomonas* as a model system. International Journal of Systematic and Evolutionary Microbiology 50:665–677.

RANKER, T. A. AND A. F. SCHNABEL. 1986. Allozyme and morphological evidence for a progenitor-derivative species pair in *Camassia* (Liliaceae). Systematic Botany 11:433–445.

RICHARDSON, J. E., M. F. FAY, Q. C. B. CRONK, AND M. W. CHASE. 2003. Species delimitation and the origin of populations in island representatives of *Phylica* (Rhamnaceae). Evolution 57:816–827.

ROE, A. R. AND F. A. H. SPERLING. 2007. Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach. Molecular Ecology 16:3617–3633.

SITES, J. W. AND J. C. MARSHALL. 2003. Delimiting species: a Renaissance issue in systematic biology. Trends in Ecology and Evolution 18:462–470.

——— AND ———. 2004. Operational criteria for delimiting species. Annual Review of Ecology, Evolution, and Systematics 35:199–227.

SMITH, J. F. AND T. A. BATEMAN. 2002. Genetic differentiation of rare and common varieties of *Eriogonum shockleyi* (Polygonaceae) in Idaho using ISSR variability. Western North American Naturalist 62:316–326.

——— AND T. V. PHAM. 1996. Genetic diversity in natural populations of the rare Idaho endemic *Allium aaseae* and its relatedness and potential

introgression with *Allium simillimum* using RAPD markers. American Journal of Botany 83:717–726.

SUATONI, E., S. VICARIO, S. RICE, T. SNELL, AND A. CACCONE. 2006. An analysis of species boundaries and biogeographic patterns in a cryptic species complex: the rotifer - *Brachionus plicatilis*. Molecular Phylogenetics and Evolution 41:86–98.

SWOFFORD, D. L. 2002. PAUP* Phylogenetic analysis using parsimony (* and other methods). Version 10. Sinauer Associates, Inc., Sunderland, MA.

TRAVIS, S. E., J. E. BAGGS, AND J. MASCHINSKI. 2008. Disentangling the role of hybridization in the evolution of the endangered Arizona cliffrose (*Purshia subintegra*; Rosaceae): a molecular and morphological analysis. Conservation Genetics 9:1183–1194.

VOS, P., R. HOGERS, AND M. BLEEKER. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

WALDRON, J., C. P. PEACE, I. R. SEARLE, A. FURTADO, N. WADE, I. FINDLEY, M. W. GRAHAM, AND B. J. CARROLL. 2002. Randomly amplified DNA fingerprinting: a culmination of DNA marker technologies based on arbitrarily-primed PCR amplification. Journal of Biomedicine and Biotechnology 2:141–150.

WAUGH, R., N. BONAR, E. BAIRD, B. THOMAS, A. GRANER, P. HAYES, AND W. POWELL. 1997. Homology of AFLP products in three mapping populations of barley. Molecular and General Genetics 255:311–321.

WHITTALL, J. B., C. B. HELLQUIST, E. L. SCHNEIDER, AND S. A. HODGES. 2004. Cryptic species in an endangered pondweed community (*Potamegeton*, Potamogetonaceae) revealed by AFLP markers. American Journal of Botany 91:2022–2029.

WIENS, J. J. AND T. L. PENKROT. 2002. Delimiting species based on DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). Systematic Biology 51:69–91.

WILSON, R. A. 1999. Species. The MIT Press, Cambridge, MA.